Testing the utility of stable isotope analysis for analyzing bee foraging patterns across habitats

Research Thesis

Presented in partial fulfillment of the requirements for graduation
with research distinction in Biology in the undergraduate colleges of The Ohio State University

by

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1. **Introduction**

Bees are the primary pollinators for most ecological regions of the world and are vitally important to terrestrial ecosystems and crop production. Approximately 75% of agricultural crop species benefit from insect pollination (Klein *et al.* 2003), which attributes to a global worth of $215 billion in food production (Gallai *et al.* 2009). There is a growing body of evidence for a steady decline in bee populations globally (Symanski *et al. 2016;* Goulson *et al.* 2015; Burkle *et al. 2013*), and these declines may directly threaten pollination services (Potts *et al.* 2010). The global decline in bee populations can be attributed to a combination of environmental stressors such as habitat loss, increased use of pesticides, exposure to parasites and diseases, reduced diversity and availability of floral resources. These factors combined negatively impact pollinator communities (Goulson *et. al* 2015).

The concern over bee declines has stimulated efforts to conserve and create bee habitat in many kinds of ecosystems including agricultural, suburban, urban, and others. For example, many countries offer financial incentives to farmers for taking measures to boost biodiversity. Some methods include sowing flower rich fields or retaining patches of natural habitat adjacent to farm land (Steffan-Dewenter *et al.* 2002). Urban areas can also support high populations of pollinators and there have been efforts to convert amenity grassland in urban areas to wildflower patches to boost wild pollinator numbers (Blackmore & Goulson 2014).

Our ability to tackle these conservation issues is limited by our understanding of bee foraging patterns and habitat requirements. Methods to determine which habitats bees utilize most often and over the course of their lifetime would help managers implement more effective conservation strategies. Unfortunately, studying bee foraging patterns using traditional methods is difficult, time-consuming, and limited spatially, temporally, and to certain bee genera (Brosi *et*

al. 2009). Direct observation has been used to monitor bee diet and foraging patterns, but can become a cumbersome, if not impossible task for bees that have large foraging ranges (e.g., up to 10 km for honey bees), are highly generalized in their foraging, have long active periods, or forage on inaccessible resources. In addition, this method only provides a snapshot of the bee diet and would require multiple rounds of observations to have a complete picture (Greenleaf *et al.* 2007). Another technique used to monitor bee foraging patterns is harmonic radar tracking. This detection system requires manually attaching a diode-antennae complex to the thorax of the bee that will directly relay signals back to a harmonic radar (Mascanzoni & Wallin 1986). While the radar system does effectively trace the foraging pattern of individual bees, the bulky apparatus requires hive entrance modification, something that is not possible for solitary ground nesting or stem nesting bees. The bulky antenna limits the usefulness of this method to only certain bee genera and is limited to open fields and would not work in forest, mountainous areas, or areas where there are buildings and trees. Another common method to analyze honeybee foraging patterns is waggle dance interpretation. Honeybee foragers possess a remarkable ability to communicate the location of floral resources to hivemates through a series of waggle and run movements, where the direction and distance is indicated by the angle and duration of the run. Scientist can interpret these movements and use them to create a spatial map of honeybee floral resources (Abbot & Dukas 2009). While effective, this technique is limited to the *Apis* genera as no other genera of bee has evolved this method of communication (Abbot & Dukas 2009). Finally, some studies remove pollen loads carried by individual bees to provide a record of the flowers they have visited over a foraging trip (Klejin & Raemakers 2008). This method provides useful information but is often too labor-intensive to carry out for studies with many pollinators and only provides a snapshot of flowers visited during the last foraging trip. An alternative

approach that could be applied to a broad range of pollinators over the course of a lifetime while remaining reasonable in terms of cost and labor would be beneficial.

Stable isotope analysis has been used in many systems to reconstruct diet from isotopic signatures of body tissues and may provide insights into the foraging patterns of bees across landscapes in a much more efficient manner. A stable isotope is an atom whose nucleus contains the same number of protons but an additional one or two neutrons compared to its lighter elemental counterpart (e.g., ^{12}C and ^{13}C) and is naturally abundant in nature (SIF 2015). These additional neutrons increase the atomic weight of the atom and cause the heavier isotopes to react more slowly in biological and physical reactions and isotopic separation, or fractionation, occurs as the molecules are incorporated into body tissues. There is reason to believe isotopic ratios of plants differ between habitats due to fractionation. For example, open field habitats are exposed to large amounts of sunlight and wind and thus are drier than forested habitats. Drying can cause the plants to close their stomata, leading to CO2 depletion in the leaf and an increase in the fixation of the heavy isotope of carbon (^{13}C) (Peterson *et al.* 1987). Plants have also been found to fractionate heavy isotopes of nitrogen (^{15}N). For example, areas exposed to high levels of drying experience increased volatilization of ammonia (NH_3) from the soil, which increases the concentration of ^{15}N incorporated in plant tissue (Nonmilk *et al.* 1994). The isotopic fractionation that results from these processes should be reflected in tissues of bees that feed on forest or open field resoures and thus provide information about bee habitat use for individuals (Hopkins 2014). If isotopic ratios of the plants differ between habitats, then individuals foraging in different habitats should have distinctive isotopic ratios. For example, a study in southern Costa Rica found a substantial difference in the isotopic signatures of bees collected in two

environmental extremes: the largest forest and the largest open pastures in the region (Brosi *et al.* 2009).

For this study, I investigated the utility of stable isotopes for tracking the habitat origin of bee nutritional resources by analyzing the carbon and nitrogen stable isotope ratios of native bee tissues collected from two adjacent habitats – the forest and an old field. In addition, I also tested differences among the isotopic signatures of bumble bees caught during two seasons when resource distributions are spread across distinct habitats: spring when flowers are mainly in the forest understory and mid-late summer when flowers are mainly located in open habitats.

2. Using stable isotope analysis to determine foraging patterns across habitat

a) Introduction

This study aimed to determine whether bee isotopic signatures are distinct across habitats. Bees were collected from two adjacent habitats: forest and old field. The plants in each habitat were expected to have a unique isotopic signature because of environmental differences that directly influence the amount of stable isotope fractionation that occurs during photosynthesis. Old field habitats experience more sunlight and drying and plants collected in this habitat should be more enriched in $\delta^{15}N$ and $\delta^{13}C$ relative to forest plants. Bees incorporate stable isotopes into their tissues from pollen and nectar collected from these plants and should reflect that in their isotopic signature. I hypothesized that bees caught in old field habitats will have higher relative concentration of $\delta^{15}N$ and $\delta^{13}C$ in comparison to forest bees. I also investigated the variation in isotopic signature across bee taxa and hypothesized that habitat would influence isotopic signature more than generic identity.

b) Methods

i) Study region

I conducted fieldwork at Dawes Arboretum (39° 58′ 44.5″ N, 82° 25′ 1.4″ W) in Newark, Ohio. Dawes Arboretum is an 1,800-acre mosaic of fields, forest, and gardens that offers a variety of resources for bees. These habitats each have their own unique isotopic signature due to variation in sunlight and wind exposure. Plants in these habitats bloom at different times of the year, attracting some bees that forage in only one habitat. These habitat specialist bees tend to be solitary species with flight periods limited to about four weeks (Zurbuchen *et al.* 2010).

ii) Bee & flower sampling

I sampled bees using aerial netting in forest and adjacent old field habitats from May to July 2014. I collected samples from 0900 to 1400 hours on fair-weather days when ambient temperatures were at least 18°C. Flower samples were collected from all the plants that the netted bees foraged on (Table 1). Flower and bee samples were stored in paper envelops and glass vials respectively and stored in a freezer.

Table 1. Sample sizes of flowers

	Habitat	
Flowers	Old Field	Forest
Packera	0	1
Claytonia	0	1
Hydrophyllum	0	2
Erigeron	1	0
Taraxicum	1	1
Geranium	0	2
Cirsium	1	4
Leucanthemum	0	1

I analyzed stable isotopes from a total of 23 bee specimens across 8 different genera: *Andrena, Augochlorella, Ceratina, Coelioxes, Heriades, Augochlora, Halictus and Nomada* (Table 2). I chose these genera because they are generalist, forage in both forest and old field habitat, and have relatively small foraging ranges. The *Nomada, Coelioxes, and Heriades* were excluded from the ANOVA due to an inadequate sample number.

Table 2. Sample sizes across genera

	Genera							
Habitat	*Andrena*	*Augochlorella*	*Ceratina*	*Coelioxes*	*Heriades*	*Augochlora*	*Halictus*	*Nomada*
Old Field	0	0	1	0	0	0	5	1
Forest	4	4	3	1	1	3	0	0

iii) Isotope preparation & laboratory work

To prepare the samples for isotope analysis, I rinsed the bees with distilled water in a vortex mixer and examined them under a dissecting microscope, physically removing extraneous particles (i.e., pollen loads, mites, etc.) using forceps. I dried the bees at 50°C for 12 hours and then ground each bee into a homogenous powder with a clean agate mortar and pestle and measured 1.00 ± 0.10 mg to package into individual tin capsules (EA Consumables Inc., Pennsauken, NJ, USA). Samples were analyzed on a Costech Elemental Analyzer coupled to a Finnigan Delta IV Plus stable isotope ratio mass spectrometer under continuous flow using a CONFLO III interface in the Stable Isotope Biogeochemistry Laboratory at The Ohio State University, Columbus, OH, USA. Approximately 10% of all samples were run in duplicate. Stable carbon and stable nitrogen ($\delta^{15}N$ = per-mil deviation of ^{15}N: ^{14}N relative to air) measurements were made where the average standard deviation of repeated measurements of the USGS40 and USGS41 standards were 0.04 per mil for $\delta^{13}C$ and 0.11 per-mil for $\delta^{15}N$.

I use the following equation to calculate C and N isotope ratios relative to standard values (d13C or d15N):

$$\delta 13C \; or \; \delta 15N \; = \; 1000 \, [(\frac{Rsample}{Rstandard}) - 1 \,]$$

where (here define any variables). R-values are the ratios of the heavy to light isotope (e.g., $^{13}C/^{12}C$ or $^{15}N/^{14}N$). The standard for expressing the isotopic ratios is $\delta^{13}C$ and $\delta^{15}N$ signifying parts per thousand (‰). These ratios are reported relative to the international standard of Vienna-PeeDee belemnite for carbon and atmospheric N_2 for nitrogen. Since these units are expressed relative to the standard, the δ value can be positive or negative.

iv) Data analysis

I performed an analysis of variance using the statistical software JMP Pro v.12 for Mac to test for effects of habitat and its interaction on two response variables: $\delta^{13}C$ and $\delta^{15}N$ isotopic signature of both bees and flower tissue. To determine what difference were present in stable isotopes among genera, a post hoc test was performed using the Turkey HSD test.

A scatterplot of $\delta^{13}C$ vs $\delta^{15}N$ was created with individual bee samples and flower means to visually analyze the data. A graph of means and standard deviations was also created for bee and flower stable isotopes of $\delta^{13}C$ vs $\delta^{15}N$. In all analysis, I interpreted *P*-values <0.05 as significant, and *P*-values of 0.05–0.08 as marginally significant

c) Results

i) Comparison of flower isotopic signature across habitat

The average isotopic ratios were higher in $\delta^{13}C$ and $\delta^{15}N$ for old field flowers compared to forest flowers (Fig. 1). An analysis of variance revealed habitats were found to be significantly different in $\delta^{13}C$ [$F_{1, 9} = 9.57$, $p < 0.05$] and not significantly different in $\delta^{15}N$ [$F_{1, 9} = 0.25$, $p > 0.5$].

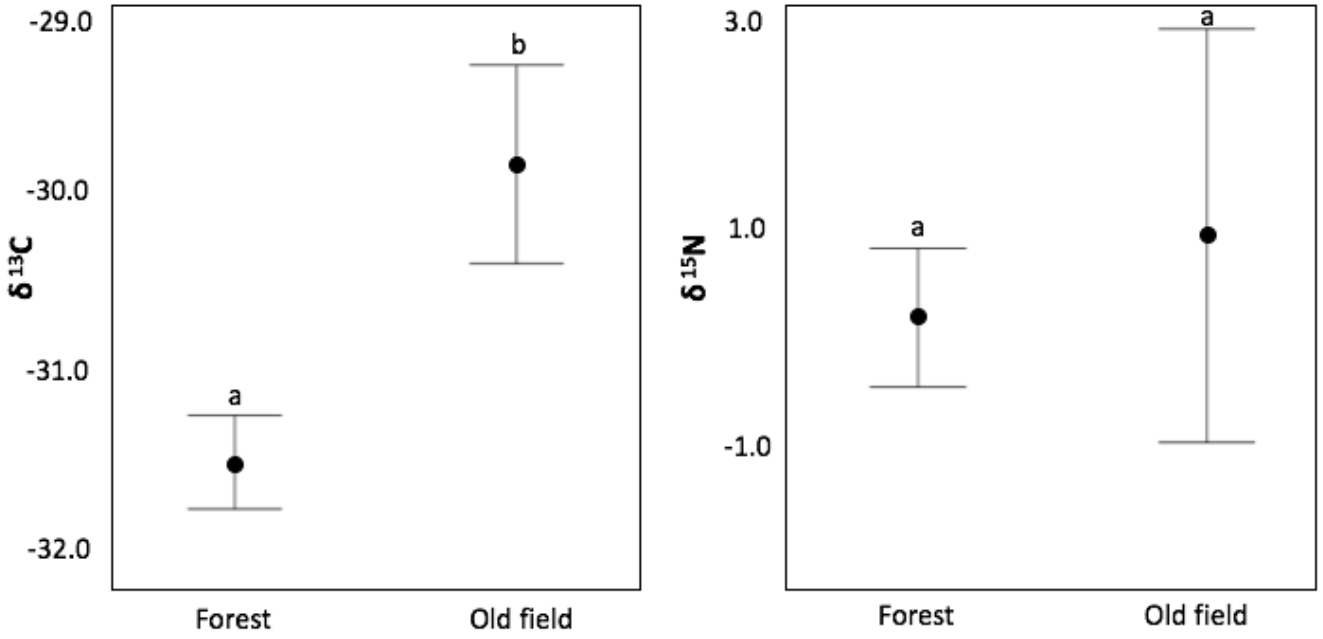

Figure 1. A comparison of flower isotopic ratios of carbon and nitrogen by habitat. The black dots represent flower means and the *error bars* are constructed from one SE from the mean (N=9). Groups not connected by same letter are significantly different.

ii) Comparison of bee isotopic signature across habitat

The average isotopic ratios were significantly higher for old field bees compared to forest bees in $\delta^{13}C$ [$F_{1, 21} = 4.36$, $p < 0.05$] and $\delta^{15}N$ [$F_{1, 21} = 24.38$ $p < 0.0001$] (Fig. 2).

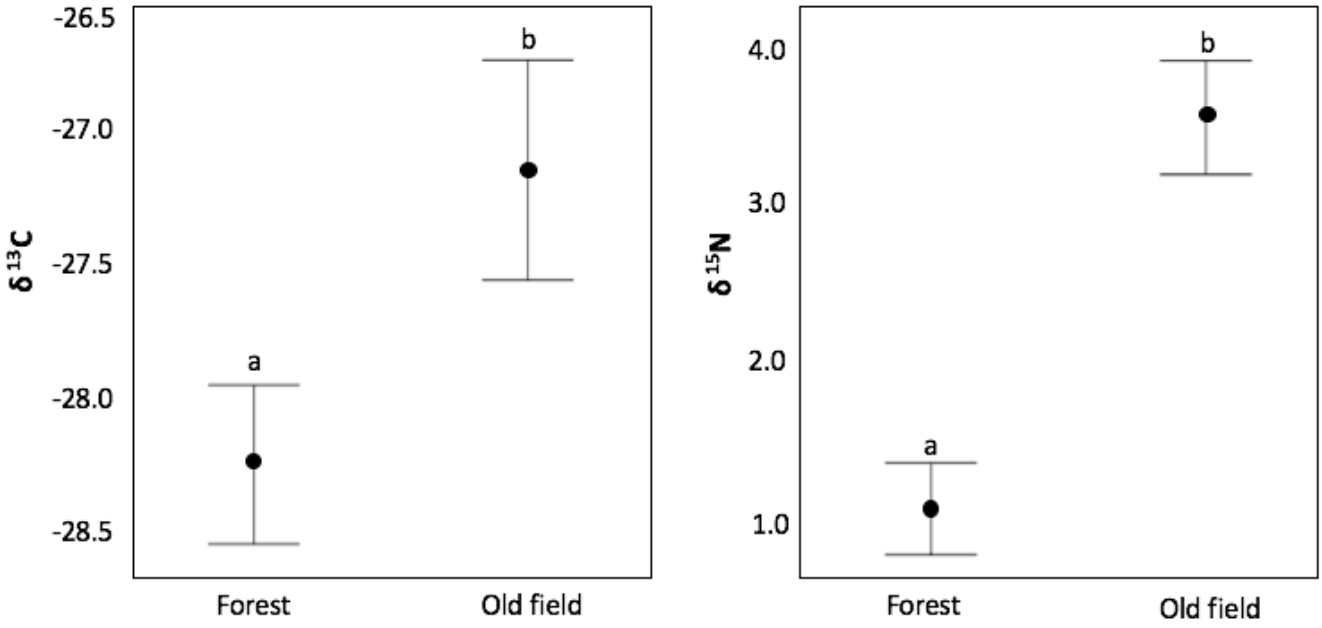

Figure 2. A comparison of bee isotopic ratios of carbon and nitrogen by habitat. The black dots represent bee tissue means and the error bars are constructed from one SE from the mean (N=21). Groups not connected by same letter are significantly different.

iii) Comparison of bee and flower isotopic signature across habitat

Forest bees tended to cluster closer to the forest flowers and old field bees near old field flowers with respect to $\delta^{15}N$ and $\delta^{13}C$ (Fig. 3). On average, old field samples were more enriched in $\delta^{13}C$ and $\delta^{15}N$ compared with respect to both flowers and bees.

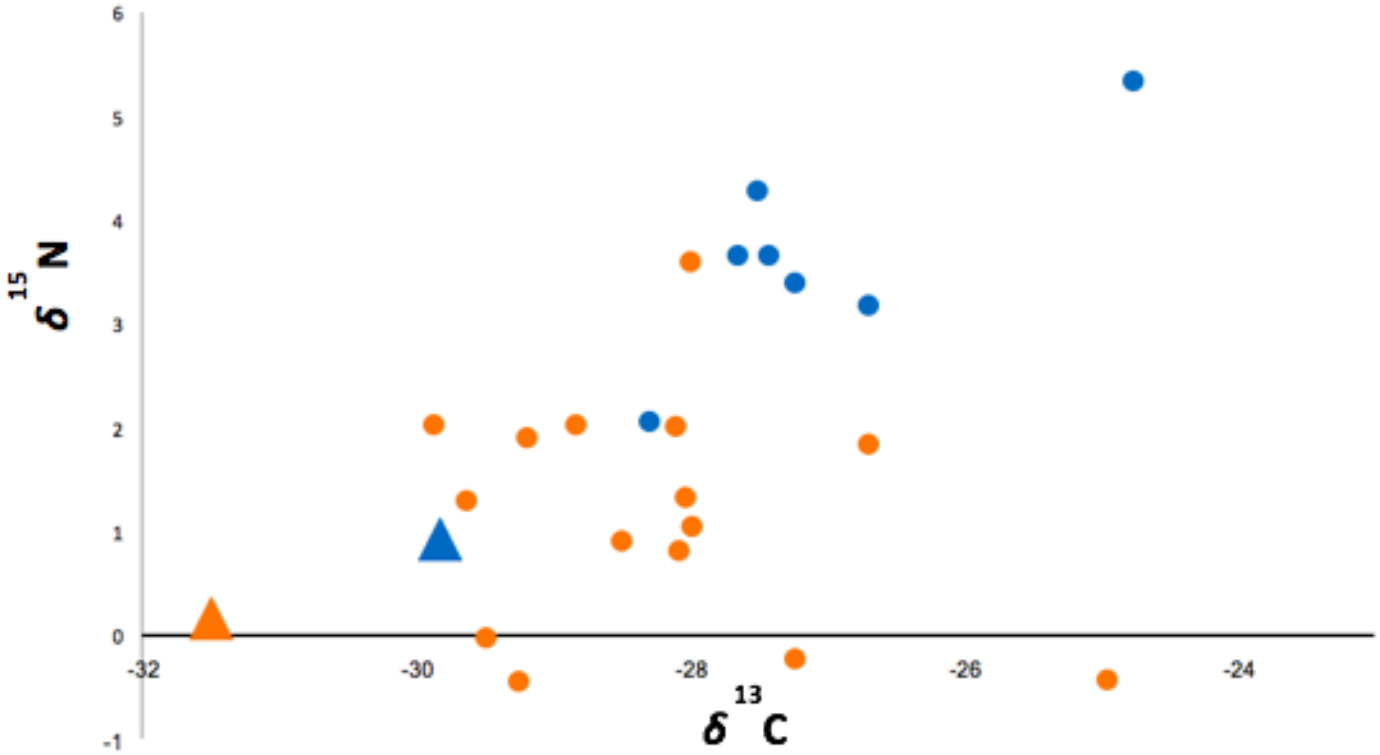

Figure 3. Isotopic ratios of individual bees represented by circles and mean ratios of flowers represented by triangles grouped by habitat. Orange are individuals collected in the forest and blue are individuals collected in the old field.

iv) Comparison of bee isotopic signature by genera

Stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ also differed significantly across genera $\delta^{13}C$ [$F_{7, 15} = 4.00$, $p < 0.05$] and $\delta^{15}N$ [$F_{7, 15} = 5.40$, $p < 0.05$]. *Andrena* and *Augochlorella* were significantly different in $\delta^{13}C$ compared to *Augochlora, Ceratina, and Halictus*. The genera that differed in $\delta^{15}N$ were *Halictus* compared to *Augochlora and Augochlorella.*

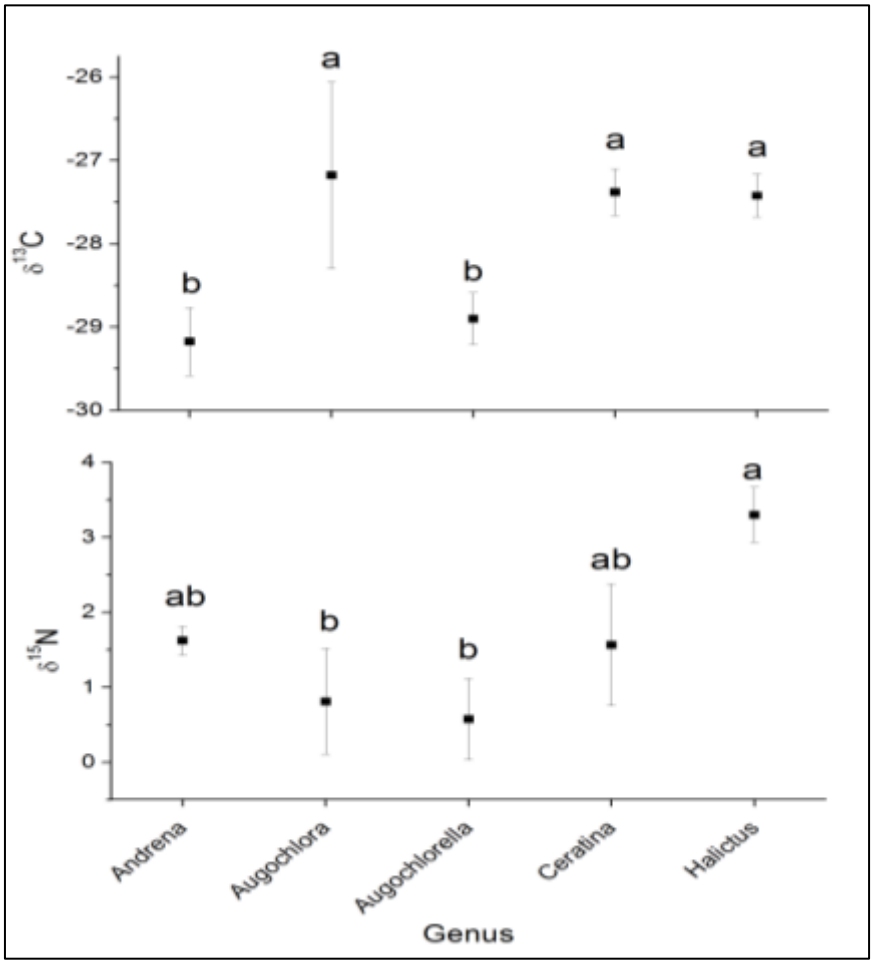

Figure 4. The black dots represent genera means and the *error bars* are constructed from one SE from the mean (N=15). Groups not connected by same letter are significantly different.

d) Discussion

The objectives were to: 1) determine if flowers collected in two adjacent, but distinct habitats differ in isotopic signature; 2) determine if isotopic signature of bees relates to flowers found in the habitat where they were collected; and 3) determine if there is variation in isotopic signature across bee taxa. I found significant differences in the $\delta^{13}C$ and $\delta^{15}N$ of plants across habitat. As expected, plants found in the old field habitat were more enriched in heavy isotopes compared to forest plants. This pattern is consistent with other studies. For example, grasslands in both China and Utah were found to be more enriched in heavy isotopes compared to shady habitats (Ehleringer *et al.* 1988; Smedley *et al.* 1991). The flowers used in this study were collected from herbaceous annual plants which may have reduced variation in the isotopic signature. Short-lived plant species such as annuals have less conservative water-use patterns than longer-lived species that have access to deeper, and more constant water sources (Smedley *et al.* 1991). This can lead to increased CO_2 depletion in the leaf and therefore more fixation of the heavy isotope of carbon. Finally, the short sample period may have also reduced variation in the flower signature. As the environment changes throughout the season, flower species respond differently and may lead to increased variation in isotope discrimination (Smedley *et al.* 1991).

There was also significant difference between the isotopic signature of old field and forest bees, indicating some potential for distinguishing bee foraging using carbon and nitrogen stable isotopes. On average, bees found in the old field had significantly higher levels of $\delta^{13}C$ and $\delta^{15}N$. However, while bees tended to cluster by habitat, there was a lot of variation among bees within each habitat. For example, many bees from the forest had $\delta^{13}C$ values very similar and some even greater than old field (Fig 3). This isotopic variation within habitat may have been caused by bees foraging outside of the study area or flowers with unique isotopic

signatures. Flowers in each habitat were quite variable so depending on which flower a bee was using, its isotopic signature may differ from the habitat mean in flowers. In addition, bees are mobile and could have potentially foraged across habitats if floral resources were available in both during their lifetime. Finally, there is likely some influence of larval diet on the bee's isotopic signature, which would reflect the isotopic values of flowers foraged on by their mothers from the previous year. The differences in $\delta^{15}N$ and $\delta^{13}C$ across habitat were consistent with results found in other stable isotope studies. For example, a study in southern Costa Rica found a substantial difference in the $\delta^{15}N$ and $\delta^{13}C$ stable isotopes of bees collected in two environmental extremes: the largest forest and the largest open pastures in the region (Brosi *et al.* 2009).

Finally, I found significant differences in $\delta^{15}N$ and $\delta^{13}C$ across genera (Fig. 4). The variation in isotopic signature may be explained by differences in dietary patterns. For example, two bees – *Nomada articulate* and *Coelioxes sayi*—held the highest values of $\delta^{13}C$ for old field and forest bees, respectively (Fig. 3). However, the enriched $\delta^{13}C$ is may not be a reflection of the habitats isotopic signature but rather that both bees are kleptoparasites. These bees enter the nest of other pollen-collecting bees and lay their eggs in cells provisioned by the host bee. When the parasitic larva emerges, it will eat the host larva along with any pollen provisions (Kreuter *et al.* 2011). The consumption of the host larva may further enrich the bee isotopic signatures in $\delta^{13}C$ and $\delta^{15}N$. There were also significant differences in the isotopic signature of genera within habitat. For example, *Augochlorella* and *Augochlora* were both collected in the forest but were found to have significant differences in $\delta^{13}C$ (Fig. 4). Conversely, *Halictus* and *Ceratina* were collected different habitats and were found to have no significant difference in isotopic signature. This suggest that genera may also be an indicator of isotopic signature irrespective of habitat and should be considered when interpreting isotopic values. However, it should be noted that this

study had a relatively small sample size and lack of overlap in genera across habitats. A future study with a more in-depth sampling is necessary.

The results of this experiment show promise for the use of stable isotopes analysis as a tool to analyze bee foraging patterns. However, many questions will still need to be answered before it is considered an effective method. For example, is the variation in isotopic signature due to taxonomy or habitat differences? This question will be explored in experiment two. The variation found within each habitat also makes it difficult to assign bees with 100% accuracy. A future study may use controlled feeding to explore how large variations in diet affect the isotopic signature of bee tissue. There was also significant variation in genera across habitat and a future study with a larger sample size may reveal more insight. Finally, stable isotopes could be used to distinguish large scale foraging patterns comparing bees from different ecosystems.

3) Examining bumble bee isotopic signature across season

a) Introduction

In this study, I investigated the isotopic signatures of bumble bees and flowers collected during three distinct time periods: spring, early summer, and late summer to determine if temporal shifts were observable in isotopic values. I conducted my research at Dawes Arboretum and used the same forest and old field habitats that I used in the previous experiment to collect samples. These habitats each have their own unique isotopic signature due to variation in sunlight and wind exposure. Additionally, these habitat's flowers bloom at different times of the year, attracting bees that only forage in one habitat. This will allow for a test to determine if there is a habitat signal in the bee isotopic signature.

I chose bumble bee workers because they have a relatively long life cycle that spans a major habitat shift in available resources. Bumble bee queens emerge in early spring and begin collecting pollen and nectar from woodland plants to provide food for their first brood of worker bees. The brood will take approximately 4-5 weeks to develop from egg to adult and emerge from the nest in early summer (Goulson *et al.* 2005). By collecting flower samples in the spring, when most flowers are found primarily in the forest, I could then analyze the larval diet of earliest bumblebee workers that emerge 4-5 weeks later. As summer approaches, flowers begin to bloom in the open grasslands and bumblebees have a new habitat for foraging. The next set of brood now feed on a diet consisting of early summer plants. Again, by collecting flower samples during early summer, when most flowers are found primarily in open grassland, I could then analyze the larval diet of the worker bees that emerge in mid-summer. I repeated this process again for mid-summer flowers that would reflect the larval diet of worker bees emerging in the

late summer. By staggering the time of flower and bee collection, I could then document the change in stable isotopes with landscape and season in context.

I hypothesized that the isotopic signature of bumble bees caught in June would match the isotopic signature of the flowers collected in the month of May. As summer progresses, the bees isotopic signature should gradually shift to heavier isotopes as herbaceous forest plants begin to die and herbaceous plants in the old field begin to bloom.

b) Methods

i. Study System

I chose two early emerging bumble bee species: *Bombus bimaculatus* Cresson and *B. impatiens* Cresson for this study. Both species are generalist foragers. The queens emerge from hibernation in April and produce female workers that persist for most of the growing season. These species likely use woodland plants in the spring, but shift their foraging to the open grasslands after the forest canopy leafs out in the summer. Therefore, this system provides a good test of the influence of habitat origin of food resources on bee isotopic signatures that controls for taxonomic variation among bees.

ii. Flower sampling

I collected flowers from have three sample periods: May-early June (early summer), late June-July (mid-summer), and August-September (late summer). In the month of May, I collected samples of flowers found in the field site to serve as a reference for larval diet of bees collected ~4 weeks later. Only flowers that bumble bees have been known to forage on were collected for analysis (Table 3). The flowers are grouped by the larval diet of the bee (e.g., spring flowers are labeled as early summer to reflect larval diet of early summer bees). Flower samples were placed in paper envelops and stored in a freezer before isotope analysis.

Table 3. Sample sizes of flowers

Early summer flowers	*N*	Mid summer flowers	*N*	Late summer flowers	*N*
Trillium grandiflorum	1	*Trifolium pratense*	4	*Prunella vulgaris*	2
Dicentra cucullaria	1	*Trifolium hybridum*	2	*Trifolium hybridum*	4
Mainthemum racemosum	1	*Achillea millefolium*	2	*Solidago spp.*	2
Rubis argutus	1	*Erigeron annus*	2	*Chamaecrista fasciculata*	1
Packera glabella	1	*Packera glabella*	2	*Melilotus alba*	2
Geranium maculatum	1	*Leucanthemum vulgare*	1	*Trifolium pratense*	1
Hydrophyllum macrophyllum	1	*Rosa multiflora*	1	*Erigeron annuus*	1

iii. Bee sampling

I sampled using aerial netting in forest and old field habitats from early June to September 2015 with a focus on bumble bees (Table 4). Each sample period was conducted over the course of one week. I collected samples from 0900 to 1400 hours on fair-weather days when ambient temperatures were at least 18°C. Bees were placed in individual glass vials and stored in the freezer before analysis.

Table 4. Sample sizes of bumble bees

	Season		
Bees	Early summer	Mid summer	Late summer
Bombus impatiens	8	9	10
Bombus bimaculatus	1	1	0

iv. Isotope laboratory work

To most accurately observe temporal shifts in the isotopic signature of the bees I used flight muscle tissue for analysis. Flight muscle is less metabolically active and reflects changes in the diet more slowly compared to other tissues (Gratton & Forbes 2006). The isotopic

signature of the flight muscle would therefore reflect long-term diet rather than the most recent meal of the bee. The flower head from each plant was also collected to gather data on the stable carbon and nitrogen isotopic ratios characteristic of each specific geographical area. I compared the isotopic signatures of bees foraging at different times throughout the season to determine whether the isotopic signatures differ. I also compared the isotopic signatures of bees to the flowers that they were collected on and the average of flowers blooming in the time period prior to their capture to determine how closely they are related.

To prepare the samples for isotope analysis, we first dried the bees and flowers in an incubator at 50°C for 18 hours. The bumble bees' flight muscle tissue was extracted after drying and ground into a homogenous powder with a clean agate mortar and pestle and measured 1.00 ± 0.10 mg to package into individual tin capsules. The entire flower head was ground into a powder and packaged into tin capsules measured at 3.00 ± 0.20 mg.

The samples were analyzed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer at the UC Davis Stable Isotope Facility at the University of California, Davis, CA, USA. Approximately 10% of all samples were run in duplicate. Stable carbon and stable nitrogen (δ^{15}N = per-mil deviation of ^{15}N: ^{14}N relative to air) measurements were made where the average standard deviation of repeated measurements of the USGS40 andUSGS41 standards were 0.02 per mil for δ^{13}C and 0.3 per-mil for δ^{15}N. For more information on how *δ-values* were calculated, see Experiment One.

v. Data analysis

I performed an analysis of variance using the statistical software JMP Pro v.12 for Mac to test for effects of time of capture and its interaction on two response variables: δ^{13}C and δ^{15}N of both bumble bees and flowers. To test for differences in stable isotopes among groups, a post hoc

test was performed using the Turkey HSD. In all analyses, I interpreted *P*-values <0.05 as significant, and *P*-values of 0.05–0.08 as marginally significant

c) Results

i) Comparison of flower isotopic signature across season

The average isotopic ratios of flowers varied slightly in $\delta^{13}C$ by season and remained relatively constant in $\delta^{15}N$ (Fig. 6). Late summer flowers differed significantly in $\delta^{13}C$ from early and mid-summer flowers [$F_{2, 34} = 8.69$, $p < 0.05$]. However, there was no significant differences in $\delta^{15}N$ among flower means for the three periods [$F_{2, 34} = 2.23$, $p > 0.05$] (Fig. 5).

ii) Comparison of bumble bee isotopic signature across season

The average isotopic ratios of bumble bees varied across season (Fig. 5). Early summer bumble bees had significantly higher in $\delta^{13}C$ compared to mid and late summer bumble bees [$F_{2, 26} = 9.06$, $p < 0.001$]. However, there was no significant difference between mid and late summer bumble bees for $\delta^{13}C$. In addition, I found that there was no significant difference in $\delta^{15}N$ across season [$F_{2, 26} = 1.72$, $p < 0.05$].

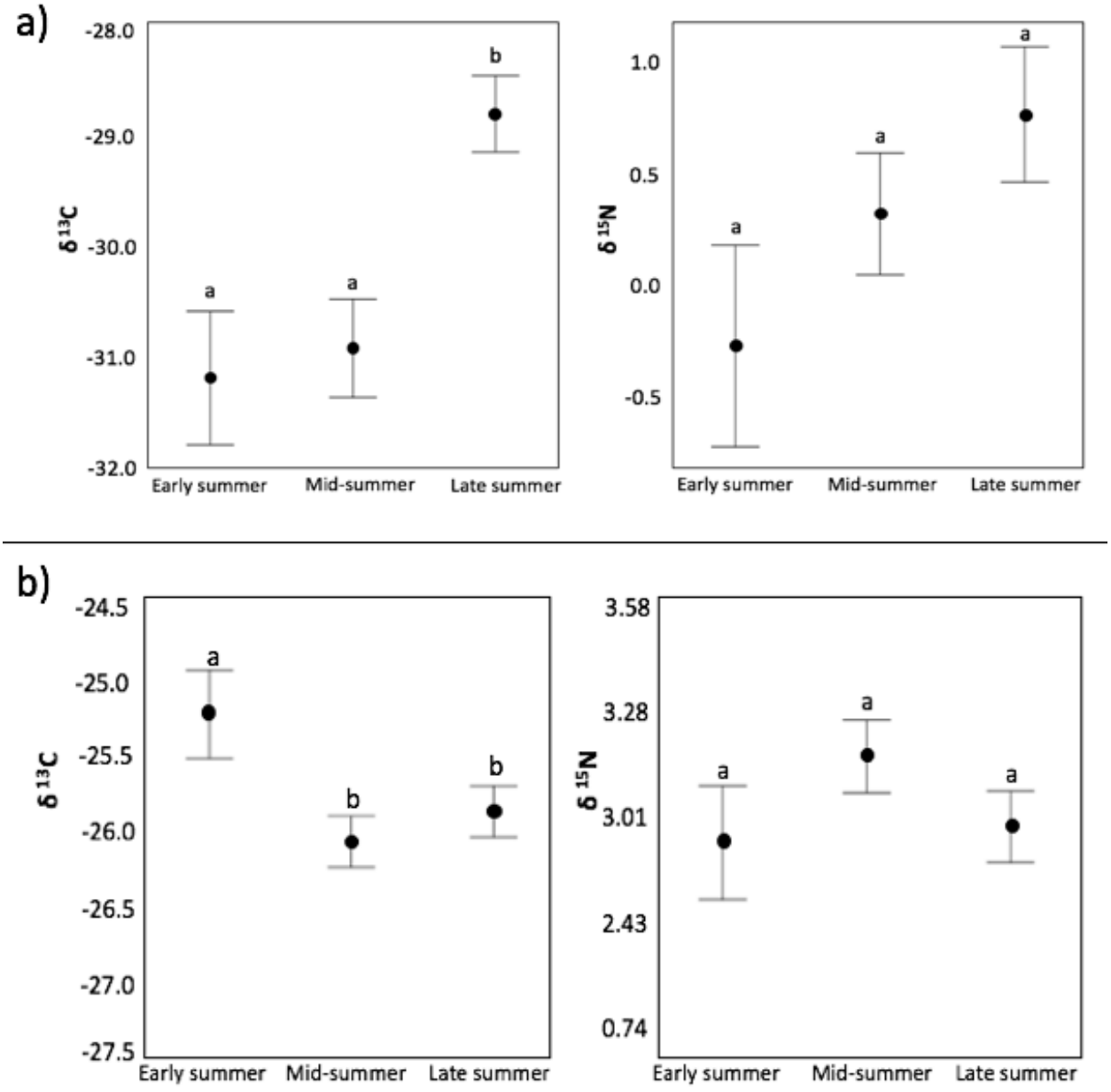

Figure 5. A comparison of flower (N=34) and bee (N=26) isotopic ratios of carbon and nitrogen by season designated as a) and b*)* respectively. The black dots represent seasonal means and the *error bars* are constructed from one SE from the mean. Groups not connected by same letter are significantly different.

d) Discussion

The objectives were to: 1) determine if flowers isotopic signature differed across season and 2) determine if bee isotopic signatures differed across season. I found that early summer bees had a much higher mean $\delta^{13}C$ signature compared to mid and late summer bees. This difference was reverse in the flowers. The early summer flowers had the lowest mean $\delta^{13}C$ of the three groups. If the bumble bees reflected their larval diet, then the early summer bees should have been expected to also be lower in $\delta^{13}C$ signature but this was not the case. Instead, the early summer bees were heavily enriched in $\delta^{13}C$. The bumble bees caught in mid and late summer also did not appear to reflect larval diet. For example, late summer bees had a similar signature compared to mid-summer bees even though the larval diet of late summer was heavily enriched in $\delta^{13}C$ compared to mid-summer larval diet (Fig. 5). Furthermore, there was no significant difference in $\delta^{15}N$ across season for both flowers and bumble bees, despite a general trend towards increasing values over the season. Additional factors likely influenced the N isotopic signature of the bees.

One possible explanation for the variation in $\delta^{13}C$ in bumble bees may be the variability in flowers. Flowers in each habitat were quite variable so depending on which flower the bumble bee was using, its isotopic signature may differ from the habitat mean in flowers. In addition, bumble bees have large foraging ranges, up to 11.6 km, and flowers from other habitats may have potentially been available (Sujaya *et. al* 2012). Furthermore, bumble bees collect nectar from some floral resources and pollen from others making it more difficult to correctly assign diet. Another factor that should be considered is the age and size of the bee. Bumble bee species exhibit worker polymorphism, where workers within a colony have different tasks based off their size. For example, the largest worker bumble bees tend to forage while the smaller bumble bees

remain in the colony and care for the brood (Couvillon *et al*. 2010). To add more variability, these foragers also have a relatively high turnover rate with approximately 29% dying every five days. It is difficult then to assign larval diet to the bumble bees caught in this study due to the great variability in age among bumble bee workers (Brian 1952).

Finally, the variability in isotopic signature may be a result of differences in carbon and nitrogen fractionation in bumble bee tissue. Nitrogen has a slower fractionation rate in the tissues of bees and is more likely to reflect larval pollen provisions due to the significant amount of tissue synthesis that occurs during development (Brosi *et. al* 2009). In contrast, carbon signatures have a relatively fast fractionation rate (Gratton & Forbes 2006) and are largely dependent on nectar consumption, which occur throughout a bee's life time. Therefore, bees collecting nectar from a plant with a unique carbon signature immediately before capture may reflect short-term feeding patterns and experience potential shifts in the $\delta^{13}C$ signal. A better understanding of how bumble bees metabolize these compounds and integrate them into their tissues may help explain the variation in isotopic signature.

Overall, there does appear to be differences in $\delta^{13}C$ across season but that difference does not appear to be caused by the larval diet. Carbon and nitrogen stable isotopes did not appear to be a useful tool to distinguish the foraging patterns of bumble bees. Future studies investigating what other factors influence isotopic signature are necessary before this method can be used reliably to determine bee foraging patterns.

4) Conclusion

The two experiments set out to test the utility of stable isotopes as a method to analyze bee foraging patterns. The first study demonstrated that bees differ in isotopic signature across habitat and showed promise for use as a method to track bee foraging patterns. However, the

second study showed that the bees' isotopic signature may be more complex than diet alone. While additional research needs to be conducted to determine what other factors influence isotopic signature, its use as a method to understand bee foraging patterns is promising. Knowing how bees use different kinds of habitats can help us optimize restoration strategies on reclaimed mines to promote bee populations and aid in bee conservation. It can help us integrate the many studies done on local foraging choices of bees (small spatial scale) with landscape-level patterns of foraging and perhaps even be used to understand dispersal patterns in bees, which is very poorly understood.

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